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NUTRITION REVIEWS

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Published Monthly at Mt. Royal and Guilford Aves., Baltimore 2, Md.

For

THE NUTRITION FOUNDATION, INC.

99 PARK AVE. NEW YORK 16, N. Y.

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Rate $4.00 per year in the United States and Canada; $4.50 in other countries.
Changes of address should be sent to The Nutrition Foundation.
Entered as second-class matter at the Post Office at Baltimore, Md.

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NUTRITION REVIEWS

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CORONARY HEART DISEASE IN AUSTRALIA

Deaths from coronary heart disease in Australia are now so common that an understanding of the causes and prevention of the disease constitutes one of the major problems facing health authorities today.

In 1953* arteriosclerotic heart disease, including coronary disease accounted for 4.9 per cent of all deaths in males. However it is especially important as a cause of death among persons during the working period of their lives. In the same year (1953) it caused more than a quarter of all male deaths in the professional, administrative, commercial and clerical occupations. In the ten year period, 1940 to 1949, the reported death rate from arteriosclerotic heart disease in age groups 50 to 54 and 60 to 64 appeared to increase by about 80 to 90 per cent for males and 21 to 70 per cent for females. As has been pointed out by W. S. Davidson (*M. J. Aust.* **1,** *584 (1953)*) for Western Australia some of this apparent increase is due to the transference of the diagnosis from myocarditis to coronary occlusion, and some may be due to a decreased mortality from other causes in these age groups. Nevertheless Davidson (1953) concluded that the true increase between 1937 and 1950 is somewhere between 41 per cent and 112 per cent for males and between 9 per cent and 52 per cent for females. J. B. Cleland (*Ibid.* **2,** *733 (1949)*) noted an increase in coronary infarction in a series of 6,000 consecutive post mortems carried out in Adelaide between the years 1920 and 1946. He thought the increase was far beyond that which might be accounted for by aging of the population.

The clinical impression of doctors as well as the evidence of vital statistics supports the view that the increase in mortality from coronary disease has occurred in all occupational groups.

Davidson (1953) studied the incidence of coronary heart disease in Western Australia from 1937 to 1950 and concluded that there was a recession in the reported numbers of cases of coronary disease during the period 1942 to 1945 in the 50 to 59 age group (a group which could not have been greatly affected by war service). As there was no evidence of a reduction in the consumption of fats in Western Australia during this period he thought it unlikely that the recession was related to diet.

Great efforts have been made to elucidate the nature of the factor or factors in Western civilizations responsible for the increase in coronary heart disease. The greatest efforts have been made to try to link diet with the disease, but physical exercise, emotional stress, and cigarette smoking are other factors which have also received attention. The dietary hypotheses which include the total fat, the essential fatty acids and the now outmoded dietary cholesterol hypotheses, are well known and have been reviewed in this journal (T. B. Van Itallie, *Nutrition Reviews* **15,** *1 (1957)*).

There appears to be no doubt that an increase in the incidence of coronary heart disease occurred in Australia from 1940 to 1949 and this increase was greater than could be attributed to better diagnosis. In view of the dietary fat hypothesis, the trends in the fat, carbohydrate, and total calorie consumption in Australia during this period have been examined (*Food and Nutrition Notes and Reviews* **14,** *41 (1957), published by the Commonwealth Department of Health*). There was no discernible trend in the consumption of fat during this period. This lack of change is also true when the

* Statistical material used in this article has been extracted from the Demography Bulletins, Bureau of Census & Statistics, Commonwealth of Australia.

consumption of animal fats plus the hydrogenated fats was examined. It appears, therefore, that the recent trend in the incidence of arteriosclerotic heart disease in Australia cannot be due to an increase in the fat consumed in the diet, or to a decreased consumption of essential fatty acids. The possibility exists however, that the high dietary fat present throughout the period studied may have prepared the population for the operation of some additional factor.

A widespread belief exists that there is a close association between obesity and coronary heart disease. An examination of the evidence in the literature shows that this belief may be fallacious.

One of the most striking ways in which Western civilizations differ from the underdeveloped countries and from the situation of, for example, 30 years ago, is in the degree of mechanization of industry, transport, and even the home. Is mechanization the factor additional to the dietary fat level which may be responsible for the increase during the past 15 years in the incidence of coronary heart disease?

The more or less steady physical toil in industry, transport and the home of past years is being replaced by long periods of relative inactivity, perhaps interrupted by fairly intense bursts of activity. For example, for many people five days of relative physical inactivity are alternated with one or two days of fairly strenuous activity. This pattern of physical activity is in sharp contrast to the walk to work and the steady laboring activity of 30 years ago, a situation which still holds in the underdeveloped countries today.

Coronary heart disease has for a long time been known to be more prevalent in the professional and administrative groups in the population. A trend in recent years, however, has been for it to become more prevalent in craftsmen. In fact Australian records suggest that there may be a steeper rise in these groups than in the professional groups. The question has been asked whether this trend is due to the decrease of physical activity which has been a feature of the mechanization of transport and industry, expecially since 1940.

The researches of J. N. Morris and others (*Lancet* **II,** *1053, 1111 (1953)*) into the incidence and severity of coronary heart disease in various occupational groups in the United Kingdom are well known. Although the findings strongly support the hypothesis that the physical activity of work was a factor in the prevention of coronary heart disease, these investigators thought that there were occupational factors in coronary heart disease other than the physical effort of the job.

It is important to realize that the exercise hypothesis does not necessarily invalidate the dietary fat hypothesis. It is possible that an investigation by G. V. Mann, *et al.* (*New Engl. J. Med.* **253,** *349 (1955)*) may show a link between the influence of diet on the serum cholesterol levels and the apparent relationship of coronary disease and exercise. These workers at Harvard found that if exercise is vigorous enough to prevent gain in weight even following the daily consumption of 6,000 calories of a diet rich in fat (approximately 175 g. per day) there is no increase in serum cholesterol. When vigorous exercise was stopped, cholesterol levels of the serum promptly increased as did body weight. Some observations leading to similar conclusions have recently been made by C. Gopalan and K. S. Ramanathan (*Lancet* **II,** *1212 (1956)*). These authors found that the serum cholesterol of officers in the Defence Forces was significantly higher than that of other ranks although the diets of each group contained approximately the same percentage of fat. Among officers of the Defence Forces the incidence of coronary-artery disease was found to be 23 times higher than among other ranks. They thought that the effect of fat intake on serum cholesterol level might well be related to the different physical exertion of the two groups. By contrast, A. Keys, *et al.* (*J. Clin. Invest.* **35,**

1173 (1956)), as a result of studies in a number of different races, concluded that the fat content of the diet had much more influence on the serum cholesterol level than physical activity per se. The authors were careful to point out, however, that their results did not imply that physical activity had no effect on cholesterol metabolism.

These investigations are obviously important and further studies along these lines should be planned to investigate the relationship between coronary heart disease and the pattern of physical activity during work and recreation in more detail.

Studies in the United Kindom (R. Doll and A. Bradford Hill, *Brit. Med. J.* **1,** *1451 (1954)*) and in the U. S. A. (E. C. Hammond and D. Horn *J. Am. Med. Assn.* **155,** *1316 (1954)*) have demonstrated a statistically significant relationship between cigarette smoking and deaths from coronary heart disease.

There is a possibility that the relationship of smoking with coronary sclerosis may not be a causal one, and that both factors may be related to a common third factor. As a leading article in the British Medical Journal (*January 8th, 1955, p. 91*) points out: "Occupation requiring only light physical work which has been shown to be associated with coronary thrombosis might, for example, provide greater opportunities for heavy cigarette smoking." Nevertheless this should not be assumed, and further investigations designed to assess the role of tobacco as a causative factor should be undertaken.

For a long time physicians have suspected that coronary occlusion seems to attack individuals who have for a long time labored under mental and emotional stress. In Western civilizations there are increasing numbers of individuals of this type. Since this pattern of life is coupled with relative physical inactivity it has not been possible in epidemiological studies to assess the importance of this factor alone. More studies to assess the possible role of the emotional factor are undoubtedly needed.

In summarizing this problem in Australia, one could say that the incidence of arteriosclerotic heart disease (including coronary occlusion) has increased in Australia since 1939. This increase has not been accompanied by any trends in the quantity or type of fat eaten by Australians. While dietary fat may be a factor in the causation of this disease the present trends in the incidence of coronary occlusion in Australia appear to be due to causes other than diet.

Marked changes in the way of life during the period studied have occurred in the physical activity associated with work and recreation. There is evidence in the literature to support the view that a change in the exercise pattern of Western Civilization may be a major factor bringing about the increasing incidence of the disease. Mental and emotional stresses are closely associated in Western civilizations with relative physical inactivity. So far epidemiological studies have been unable to assess the relative importance of exercise as compared with mental and emotional stresses as etiological factors.

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MECHANISM OF SERUM CHOLESTEROL LOWERING BY SEED FATS

There is ample evidence that in individuals on a formula diet, the addition of a saturated fat results in an increase in serum cholesterol concentrations, while addition of a highly unsaturated fat has the opposite effect (*Nutrition Reviews* **13,** *8 (1955)*; **14,** *107, 327 (1956)*; **15,** *1, 39 (1957)*). However, the mechanism by which this effect is brought about has remained obscure. The possibility had to be considered that removal of the

cholesterol from the blood was simply the result of its being deposited in some other tissue, an effect which would be distressing to those using such regimen to prevent atherosclerosis. A more likely explanation, especially in view of the known highly unsaturated nature of the fatty acids of the cholesterol esters, was that in this form cholesterol could be readily solubilized and transported to the liver for excretion.

In order to test this possibility H. Gordon, B. Lewis, L. Eales and J. F. Brock (*Nature* **180,** *923 (1957)*), performed studies with human patients in which analysis of fecal steroids was carried out concurrently with dietary fat alterations and serum cholesterol analyses. Particular attention was paid to the bile acids because of the demonstration that these are the main excretory products of cholesterol (M. D. Siperstein, M. E. Jayko, I. L. Chaikoff and W. G. Dauben, *Proc. Soc. Exp. Biol. Med.*, **81,** *720 (1952)*). In the first study, four subjects, after 12 days on a basic diet (2000 calories including 8 g. fat), were divided into two groups which were maintained for 12 to 15 days on the diet in which either 100 g. hydrogenated coconut fat or 100 g. sunflower seed oil (I. N. = 135) had been isocalorically substituted. At the end of this period, the fats given the two groups were reversed. In the second study, three subjects, after ten days on the basal diet (in this case, 2500 calories) were given, in the same manner, 75 g. of hydrogenated coconut oil daily. After a further ten days, an additional 75 g. of sunflower seed oil was added to the diet (by isocaloric substitution), and after 20 days the sunflower seed oil was withdrawn and the subjects continued on the saturated fat for ten days.

In every case, as expected, the inclusion of the coconut fat resulted in increased serum cholesterol values, while the sunflower seed oil brought about decreases in serum cholesterol below that on the basal diet when it was the sole fat present.

Fecal lipid and neutral sterols increased markedly on the hydrogenated fat diet and again when the sunflower seed oil was either added or substituted. However, fecal bile acids did not change appreciably on the hydrogenated fat diet but almost doubled when the sunflower seed oil was added or substituted.

On the basis of these findings, the authors advance the tentative hypothesis that the effect of the unsaturated fat (in this case, sunflower seed oil) on the serum cholesterol levels is due to a promotion of sterol catabolism and excretion, particularly in the form of bile acids. It seems likely from this and other considerations that the low-melting polyunsaturated fatty acids, when esterified to cholesterol, promote its emulsification and incorporation into serum lipoproteins and its transport to its destination, in this case to the liver for oxidation and excretion.

LYSINE SUPPLEMENTATION IN GERONTOLOGY

It is inevitable that the ready availability of a specific nutrient should elicit considerable work aimed at determining the value of the nutrient when added to various diets. Although it is not necessary first to establish the presence of a dietary deficiency for that nutrient, it is desirable to plan a study in which the dietary supplement given is compared with that of a control group receiving exactly the same treatment except for the use of a placebo in place of the dietary supplement. Ideally, the placeboes and the supplements should be such that neither the investigators directly involved in the study, nor the subjects, are aware of the makeup of the groups. Difficulties of pairing experimental subjects with control individuals on the basis of age, size, heredity, and health status are obvious. Because of this, it is frequently necessary to use the subjects as their own controls.

More diligent application of these prin-

ciples of experimental design would have improved the study recently reported by A. A. Albanese, R. A. Higgons, L. A. Orto and D. N. Zavattaro (*Geriatrics* **12,** *465 (1957)*). These workers claim they have evidence for the improvement of nitrogen balances in elderly women, who were healthy or convalescing from surgery or an illness, by adding 600 to 900 mg. of lysine to their daily diet.

These workers calculated the lysine to tryptophan ratio of 568 meals served, over a six-year period, to 36 elderly women in an old peoples' home. This ratio averaged 4.5, compared to a value of 6.0 which was reported to be that of tissue protein. In some diets the ratio was calculated to be below 4.0. The average daily caloric intake for these women was 1775, which provided them with 59 g. of protein, 23 of which were derived from meat.

On the basis of the above calculations, and the data from nitrogen balances carried out on these women over a five-year period, lysine was added to the diets of 20 elderly women. The lysine was given in dosages of 200 to 250 mg. with each of the three daily meals for a period of 30 days or more. The nitrogen balances were repeated, presumably at the end of the supplementation period. The balances were carried out over a two or three-day period by determining the nitrogen in the excreta and calculating the nitrogen intake "from individual diet records."

Shifts in nitrogen balance in a positive direction occurred in eight of the 20 subjects. Twelve of the subjects failed to exhibit significant shifts in nitrogen equilibrium. Of these 12, nine were reported to be in relatively high positive balance in control and experimental periods alike, while three were in negative balance throughout.

The significance of the results are open to question in so far as the need for lysine supplementation of adult diets is concerned. Data presented in one of the tables on 18 to 25 nitrogen balance periods carried out on four elderly women, of ages 68 to 79 years, gave daily nitrogen retentions ranging from 0.70 to 2.45 g. for each subject. The average for the four women was 1.86 g. of nitrogen per day, which, when translated into body tissue, would represent an increase in body weight of 1.4 kg. per month. Since nothing was stated about any increase in body weight, it may be inferred that the weights were probably fairly constant. Further suggestive evidence for the stability of the body weights comes from the relatively low caloric intake. For the total group of 200 women the calorie intake averaged 1775, of which the group of 20 studied in the lysine supplementation experiment averaged 1560.

Patients in a convalescent hospital were used for the second phase of the study. These patients were recovering from a variety of diseases and a number of operative procedures. Seven to ten days after admission the subjects entered the precontrol periods of observation. In no case do the investigators give the period intervening between the acute phase of the illness and the initiation of the study. This information would have been of considerable importance since the investigators indicate their results provide evidence for the well-established fact that "during the catabolic state which accompanies severe acute illness or trauma, a negative nitrogen balance may occur . . ."

The information on the clinical condition of the patients would have been important, since each patient was studied for a one-week control period which was then followed by a week during which the patient's diet was supplemented with a tabletted preparation containing lysine and a mixture of vitamins. A "post-control" period followed the lysine period. In the pre- and post-control periods, the patients received placebo tablets containing only a mixture of vitamins. In these studies the nitrogen balance was again carried out by analyzing the excreta and calculating the intake from dietary records. The balance period covered the last two days of each week.

The nitrogen balances indicated that 18 of the 39 convalescing subjects were in

negative nitrogen balance while consuming an average of 98 g. of protein per day. Lysine supplementation at the level of 600 to 900 mg. per day improved the nitrogen balance when the daily protein intake was less than 1.5 g. per kg. body weight, or when meat protein comprised less than 30 per cent of the total protein intake.

Examination of the data presented for "16 patients which are representative of the results found for all 39 convalescent patients" indicated that during the second week of the study, when lysine was given, the nitrogen balance was better than that in the control week. However, the nitrogen balance during the post-control week when no lysine was given was as good as, if not slightly better than, that during the lysine supplementation. To what extent the general improvement of the nitrogen balance presents an improvement in the patient's condition is difficult to say, in view of the fact that proper controls were not included. Since all of the patients showed a progressive improvement in the nitrogen balance during the period of the study, a randomized group should have received placeboes throughout the entire experimental period in order to evaluate the general course of the nitrogen balance in these convalescing patients.

Graphical representation of the data for five subjects not listed in the preceding suggests that there was a progressive increase in the nitrogen intake in each of the three weeks of the study. This was associated with an increased caloric intake which was especially prominent during the "post-control" week when the lysine supplementation was discontinued.

The authors recognize that the natural "process of recovery" might explain all of their results. They claim that "long experience both at the clinical and laboratory level leads us to believe that lysine supplementation of the diet of nutritionally substandard adults constitutes an effective physiologic means of improving the efficiency with which food protein is utilized." One must recognize that clinical evaluation is a very important means of ferreting out leads for experimental studies. However, in such a commonly used procedure as a nitrogen balance, there are well established guide lines which should permit unequivocal evaluation of the data secured. It is difficult to see how a diet record could ever be accepted as a basis for quantitatively determining the nitrogen intake. To do this properly would require the weighing of all food consumed by the subject and a collection of an exact duplicate of the food intake for analysis of its nitrogen content. Until such precautions are taken, it seems premature to suggest that the primary amino acid deficiency in the diets of these older people was lysine.

Furthermore, the lysine to tryptophan ratio is not the only factor which will determine whether a diet is deficient in lysine. A ratio such as the above cannot have any significance unless it is related to the absolute amount of lysine in the diet. Recent experimental work has confirmed the fact that the minimum lysine requirement for maintenance of the non-convalescent adult, especially for women, is between 0.4 and 0.8 g. per day (*Nutrition Reviews* **15,** *303 (1957)*). It is unfortunate that the investigators did not present the data on the lysine intake, especially after going to the trouble of making the calculation for the determination of the lysine to tryptophan ratios. It appears that this could readily have been done, as Albanese and co-workers (*loc. cit*) provide data on the average percentages of various types of proteins included in the protein intake of convalescent patients on their hospital diet. Estimates of requirements of lysine and other amino acids for a satisfactory rate of convalescence would seem to be an important objective in studies of this kind.

Lysine supplementation could be expected to improve only those diets low in lysine. Classically these would be diets high in

wheat or rice, as the proteins of these cereals are particularly low in this amino acid. Typical American diets with their generous intake of animal proteins undoubtedly provide sufficient lysine. Diets high in cereals may be benefited by lysine supplementation, though this has not as yet been demonstrated in man.

The Food and Nutrition Board of the National Research Council (*Pub. Health Reports* **72,** *469 (1957)*) in a statement entitled "Supplementation of Dietary Proteins with Amino Acids" concluded as follows: "The benefits to be derived from amino acid supplementation are uncertain . . . The Food and Nutrition Board recognizes the potential value of proper supplementation with amino acids and the desireability of intensive study of the problem." Further and more carefully controlled studies will be needed before definite conclusions can be drawn regarding the value of lysine supplementation in the diets of oldsters and convalescents.

NONESTERIFIED FATTY ACID METABOLISM IN DIABETES MELLITUS

The metabolic alterations noted in patients with diabetes mellitus have been reviewed earlier (*Nutriton Reviews* **11,** *312 (1953)*; **13,** *115 (1955)*; and **14,** *196 (1956)*). These include, in addition to the abnormality in carbohydrate metabolism, disorders in the handling both of lipids and proteins. Earlier work with patients having diabetes mellitus concerned itself mainly with the role of glucose metabolism. Lipid metabolism has been investigated less enthusiastically, probably because of less definitive methods used in lipid analysis. However, abnormalities in lipid metabolism have been well documentated in patients with diabetes mellitus. Ketosis is an abnormality noted in the acute phase of the disease, and the increased incidence of atherosclerosis and coronary occlusions are evidence of the chronic effects of abnormal lipid metabolism.

Recently E. L. Bierman, V. P. Dole and T. N. Roberts (*Diabetes* **6,** *475 (1957)*) have reported studies on the nonesterified fatty acid metabolism in both normal individuals and patients with diabetes mellitus. This lipid fraction includes, in addition to the free fatty acids present in plasma, fatty acids which are firmly but reversibly bound to the serum proteins. To determine the nonesterified fatty acids, Bierman and his colleagues extracted these substances from the plasma and estimated the amount of fatty acid by titration. The method they used consisted of extracting one volume of plasma, first with five volumes of the extraction mixture (40 parts isopropyl alcohol, 10 parts of heptane and 1 part 1 N. sulfuric acid), then with a mixture of hexane and water. The solvent layer was removed and an aliquot was titrated with 0.018 N. sodium hydroxide, using an alcoholic solution of thymol blue as the indicator. The titration was carried out using a stream of carbon dioxide-free nitrogen as a mixer, so that the aqueous sodium hydroxide and solvent layers were in intimate contact.

The nonesterified fatty acid level in the plasma of 40 non-diabetic, non-obese subjects averaged 572 $\pm$ 280 microequivalents per liter (mean $\pm$ standard deviation). The average level of this lipid fraction in the plasma of 27 patients with diabetes, but without ketosis, was higher than that noted for the normal individuals, 849 $\pm$ 287 microequivalents per liter. This value was statistically different from the average noted in the non-diabetic individuals ($P < 0.001$). In ten diabetic patients who had ketonuria, the average nonesterified fatty acid level was still higher than that of the controlled diabetic individuals, 1,211 $\pm$ 466 microequivalents per liter. The nonesterified fatty acid level of obese patients was similar to that noted in the controlled diabetic

patients, 862 ± 214 microequivalents per liter. The wide limits found in any one of the groups led the investigators to conclude that this fraction of the blood lipids is very labile, and that the conditions under which it is determined must be rigidly standardized.

Giving glucagon to patients accelerates the rate of breakdown of liver glycogen and increases the blood sugar concentration. This is equivalent to subjecting the patient to an endogenous glucose load. Fourteen non-diabetic and nine diabetic patients were given 2 mg. of glucagon intravenously, and the levels of blood sugar and plasma nonesterified fatty acids were determined at intervals during the two hour period which followed the injection of the hormone. As expected there was an increase in the blood sugar level in both groups of individuals, but the blood sugar returned to the pre-injection range in about one hour in the non-diabetic patients. The diabetic patients still had elevated blood sugar levels (average 25 per cent above control values) two hours after the glucagon was given. The nonesterified fatty acid level of the diabetic individuals was depressed only about one fifth of the initial value at the end of the first hour after giving the glucagon.

During this time the non-diabetic individuals had a 50 per cent decrease in the plasma nonesterified fatty acid level. At the end of the second hour there was no further change in the level of this lipid fraction in the case of the diabetic patients. In the non-diabetic subjects the nonesterified fatty acid level of the plasma increased, reaching three-fourths of the control value by the second hour. The differences in the nonesterified fatty acid fraction in the plasma of the diabetic and non-diabetic individuals following the administration of the glucagon was significant ($P < 0.001$) at both the 40 and 50 minute periods.

Thus, the diabetic and the non-diabetic individuals showed quantitatively different responses to an endogenous glucose load, both in the blood glucose and plasma nonesterified fatty acid levels. An oral glucose load was given in the form of 50 g. of glucose to three diabetic, and 17 normal, individuals. In data obtained from a diabetic patient given 50 g. of glucose, the decrease in the nonesterified fatty acid level of the plasma was slower than that for the normal subject, but the total decrease was similar for both individuals.

Nonesterified fatty acid levels were determined in the plasma of eight patients with diabetic acidosis, and markedly elevated values were found in the fasting samples. On administration of insulin there was a prompt fall in the level of both glucose and nonesterified fatty acids. In a period of three hours, during which 50 units of insulin had been given, the blood nonesterified fatty acid level in a patient with acidosis fell from approximately 1400 microequivalents per liter to a normal value of about 400. The blood sugar had decreased from about 375 to approximately 100 mg. per 100 ml. during the same period. While the plasma nonesterified fatty acid level fell to the normal range the urine ketone level decreased from four plus to one plus, and the urinary glucose remained four plus during the entire time.

In another patient with diabetic coma, studies for a five-hour period, during which 275 units of insulin were given, the blood sugar fell from approximately 380 to about 120 mg. per 100 ml. and the nonesterified fatty acid level decreased from about 550 to 100 microequivalents per liter. The rate of decrease for this lipid fraction was greater than that for the blood sugar. The urinary ketones remained four plus during the entire period, and the urine sugar level decreased to three plus during the last hour. Thus the decrease in blood nonesterified fatty acids was correlated with the fall in blood sugar and preceded the decrease of ketonuria by several hours. These investigators have noted that the nonesterified fatty acid fraction of the blood had an exceedingly

rapid turnover rate, and it appears that this fraction has an important place in the transportation of fatty acids from the depots to the liver and peripheral tissues.

Thus the elevated levels of nonesterified fatty acids in the plasma of the diabetic patient indicates, according to these studies, an impaired control in the release of fatty acids from the fat depots. This, the investigators believe, is caused by an impaired utilization of glucose in the cells of the adipose tissue. The view that an increased release of fatty acid occurs, rather than a decreased rate of removal, is substantiated by the workers' finding that, when insulin was given, there was no increase in the rate of disappearance of injected tagged fatty acids bound to homologous albumin.

In the case of ketosis it is visioned that the body is flooded with fatty acids to such an extent that the ability to oxidize them is exceeded. Upon giving insulin, the output of nonesterified fatty acids from the depots is decreased, thus removing the cause of the acidosis. However, since the ketone bodies are oxidized slowly, the disappearance of the accumulated ketone bodies is more gradual. The use of nonesterified fatty acid level in plasma as a tool for the study of lipid metabolism may provide interesting data when employed to study other situations involving abnormal metabolic reactions.

URINE PROTEOLYTIC ACTIVITY

A proteolytic enzyme present in urine which is active at the pH range suitable for pepsin was noted by E. Brucke in 1861. During the past ten years there has been a renewal of interest in this enzymatic activity as a possible tool for both investigative and diagnostic work.

Recently H. L. Segal *et al.*, (*Gastroenterology* **33,** *557 (1957)*) have reported studies on uropepsin (proteolytic activity in the urine determined at pH 1.5). These investigators have carried out 1,189 tests on 386 subjects. Their aim was first to determine the shortest representative urine collection period which could be used, and secondly to evaluate the role of uropepsin in the differential diagnosis of various disease states. The procedure used in determining uropepsin activity was a modification of the technique reported by I. A. Mirsky, S. Block, S. Osher and R. H. Broh-Kahn (*J. Clin. Invest.* **27,** *818 (1948)*), in which the enzyme is allowed to act on a bovine hemoglobin substrate maintained at pH 1.5 and 37.5°C. for 30 minutes. The digestion of the protein liberates tyrosine-like substances, which are quantitated colorimetrically. A unit of uropepsin activity has been defined as the amount of activity which will liberate 0.1 mg. of tyrosine-like substances under these conditions. The activity per ml. of urine is multiplied by the total urinary volume and divided by the number of hours over which the urine is collected; thus, the values are expressed as units per hour.

The 386 subjects studied were arbitrarily divided into seven groups, consisting of one group of persons with no disease and six groups of patients distributed according to the following disorders: duodenal ulcer, gastric ulcer, gastric cancer, other disorders of the gastrointestinal tract, pernicious anemia, and other diseases not associated with the gastrointestinal tract.

By comparing the average uropepsin levels of urine, collected for an eight hour over-night period, to the one and three hour morning urine collections, it was noted that the two shorter periods showed higher average uropepsin levels for the various diagnostic groups. The range of the average values of the uropepsin in the eight, three and one hour urine periods for all groups studied, except for those with pernicious

anemia, were 24 to 80, 49 to 107 and 63 to 248 units per hour, respectively. These investigators believed that for comparative purposes the urines collected for eight to 12 hours were preferred because of greater consistency of values. There was extensive overlapping of the uropepsin activity among the patients with different disorders. This indicates that a single determination of the uropepsin activty is probably of no critical diagnostic use.

On analysis of these data statistically, two sources of variability were noted. For a single individual, the day to day variability was found to be 29.6 units; among the individuals in a single diagnostic group (except those with pernicious anemia) the variability of excretion rate was 38.2 units. While it was noted that the average excretion of uropepsin by individuals with peptic ulcer (80 units per hour for the eight hour period) was above that for the normal individual (54 units per hour), because of the variability of the values obtained from the same individual a single determination of the uropepsin level will not aid in differentiating this disorder from other disorders where there is free acidity in the stomach.

On the other hand, these investigators believe that the absence of uropepsin activity is of some aid in differentiating the achlorhydria of pernicious anemia from that of other achlorhydrias. They believe the presence of uropepsin activity also suggests the possibility of gastric carcinoma in certain patients with ulcerated lesions of the stomach. On checking the uropepsin excretion in patients with achlorhydria, as well as in those with free hydrochloric acid present in the stomach, it was found that the largest number of patients with low uropepsin levels were in the achlorhydric group. Usually those with high gastric acidity also had the highest uropepsin activity in the urine. Though six per cent of the patients with achlorohydria of the non-pernicious anemia type excreted large amounts of uropepsin, of the 23 patients with pernicious anemia 17 excreted no uropepsin, and the remaining five patients excreted less than 15 units per hour.

In a companion work, L. L. Miller, H. L. Segal and E. Plumb (*Gastroenterology* **33,** *566* (*1957*)) studied the laboratory factors which influenced the determination of the uropepsin levels and also obtained some information concerning the urine proteolytic enzyme. They noted that dialysis of the urine against distilled water did not reduce the activity of the urine proteolytic activity. They also found that the urine may be stored at 4°C. in the presence of toluene for one to two weeks without changing the uropepsin levels, providing there is no gross contamination with bacteria. By extracting filter paper with trichloracetic acid of the strength and amounts used in carrying out precipitation of the hemoglobin substrate, it was found that a chromogen could be leeched from the paper. This amounted to 0.34 to 0.52 units of uropepsin activity per ml. of urine. Thus, for a ten hour urine collection of 1000 ml. volume this error would amount to 34 to 52 units per hour. Using centrifugation in the preparation of the protein free filtrate they eliminated the filter paper and prevented these false high values.

In studying the proteolytic activity of 93 samples of gastric juice obtained from 30 patients, these investigators carried out studies with the substrate buffered at the pH levels of 1.5, 3.0 and 3.5. In addition a few studies were carried out by varying the pH by 0.5 units between the range of 1.5 to 4.5. In general it was noted that the proteolytic activity of the gastric juice was about the same at any pH studied between 1.5 and 3.0, but when the pH was increased to 4.0 or 4.5 there was little or no proteolytic activity. The activity at pH 3.5 was always less than that noted at more acid ranges.

The proteolytic activity of 300 urines, from both normal individuals and patients with various disease categories discussed

earlier, was determined at the pH levels of 1.5, 3.0 and 3.5. Thirteen additional samples were determined at half pH steps between 1.5 and 4.0. As found in the case of gastric juice, the greatest proteolytic activity was noted in the pH range of 1.5 to 3.0. At 3.5 the proteolytic activity of the urines varied considerably, and not infrequently exceeded the values noted at pH 1.5.

These investigators also studied the possibility that uropepsin might be excreted in the urine in an inactive form, uropepsinogen. In these investigations the urine was first incubated at pH 1.5 before the addition of the substrate. This treatment would be expected to activate the uropepsin precursor, uropepsinogen, if it were present. No increased activity was noted following this treatment. On the other hand, a preliminary incubation of the urine at pH 3.5 for one hour was required to achieve maximum proteolytic activity at this pH. The activation at pH 3.5 is difficult to explain, and it is inconsistent with the known facts for the conversion of pepsinogen to pepsin. The possibility of substances which inhibit the proteolytic activity in the urine was considered, but since there is practically no knowledge on this subject no further information can be offered.

The ratio of proteolytic activity as determined at pH 3.5 to 1.5 was determined in the gastric juice obtained from two normal male subjects. These individuals were connected to constant suction apparatus which removed the gastric secretion by means of an indwelling Levine tube. The tube was inserted in the evening and collections continued until 6:00 p.m. of the next day. During this time the patients each received two separate injections of 0.75 mg. of histamine diphosphate which stimulated gastric secretion. A lack of correlation was noted between the pH of the gastric juice and the activity ratios (activity at pH 3.5/activity at pH 1.5). The activity measured at pH 1.5 varied greatly from specimen to specimen (227 to 1280 units per min.) and so did the activity found at pH 3.5 (0 to 394), but no relationship was found between the activities noted at these two pH levels. It was observed that histamine stimulation increases the proteolytic activity at pH 1.5 but not at 3.5. Urine specimens were collected at 12 hour periods during the time the gastric juice was being tested, and it was found that the activity in the urine as determined at either pH 1.5 and 3.5 was constant. The activity ratio determined in two urine periods were 0.6 and 0.9, while that in the fasting gastric juice was in the neighborhood of 0.16, indicating that considerably more activity occurred at pH 3.5 in the urine than in the gastric juice.

These investigators postulated that gastric pepsin (determined at pH 1.5) and gastric cathepsin (determined at pH 3.5) are two separate enzymes and they are secreted into the stomach and excreted into the urine at different rates. Also there was no direct correlation between either the gastric cathepsin or urocatheptic activity with a variety of disease states or different degrees of gastric secretory activity. The value of a single determination of urine proteolytic activity in the diagnosis of diseases is seriously questioned because of wide variation in results. The exception is that urine proteolytic determinations may be of some value in indicating the presence or absence of achylia.

The proteolytic activity present in the urine which is active at pH 1.5 has been known for a long while, but further work on the characterization of the enzyme or enzymes has been neglected, probably because much of the early work was confined to seeking clinical applications for this activity. Now more fundamental studies on this series of enzymes has been started, and through such investigations a better understanding of these catalysts will be brought about. This in turn may allow for clinical uses not foreseen at present.

VITAMIN D DEFICIENCY IN THE CAT

The nutritional requirement of cats has not been widely investigated. Several groups of workers have reported that cats appear to require high fat diets, that is, diets containing 25 to 30 per cent of fat (A. C. Da Silva, *Acta Physiol. Latinoamerica* **1,** *26 (1950)*; W. A. Krehl, G. R. Cowgill and A. D. Whedon, *J. Nutrition* **55,** *35 (1955)*; S. N. Gershoff, S. B. Andrus, D. M. Hegsted and E. A. Lentini, *Lab. Investig.* **6,** *227 (1957)*). The latter workers also noted that vitamin A absorption in cats was related to the fat content of the diet and that cats are unable to convert carotene to vitamin A.

In a recent paper these workers have investigated the effect of vitamin D-deficient diets in studies with kittens (Gershoff, M. A. Legg, F. J. O'Connor and Hegsted, *J. Nutrition* **63,** *79 (1957)*).

Three- to six-month-old kittens of mixed sexes were used. Routine pre-experimental treatment included dusting with insecticide, deworming, and vaccination against feline distemper. Only the animals which adapted to subsistence on the high-fat, high-protein, purified diet during a two-week initial trial were used in the experiment.

The purified diet had the following percentage composition: casein, 32.1; corn oil, 13; hydrogenated fat, 13; salt mixture (deficient in Ca and P), 1.8; choline, 0.3; sucrose, 36.4; and dicalcium phosphate, 3.4. This diet provided a 1:1 ratio of Ca:P. A modification of this diet, in which part of the dicalcium phosphate (1.6 per cent) was replaced by calcium carbonate (3.7 per cent), provided a Ca:P ratio of 2:0.65.

Both diets were fortified with the following vitamins (milligrams per kilogram): thiamine, 4; riboflavin, 8; niacin, 40; calcium pantothenate, 20; pyridoxine, 4; folic acid, 1; biotin, 0.2; and menadione, 1. The diets also contained added vitamin A (25,000 I.U. per kilogram). Control cats on each of the basal diets received 250 I.U. of vitamin D_3 orally twice each week.

The diet containing a Ca:P ratio of 2:0.65 was poorly or variably accepted so that only ten of 17 kittens survived on this diet beyond the first six weeks of the experiment. Twelve of 13 kittens survived the six-week period on the diet with a Ca:P ratio of 1:1.

Most of the surviving cats receiving the vitamin D-deficient diets developed rickets in four to five months. The diet with a 1:1 Ca:P ratio produced the more severe deficiency symptoms, probably because of its higher acceptability and resultantly more rapid body weight gains.

Alkaline phosphatase concentration in blood serum proved to be the most sensitive chemical means of evaluating the severity of rickets. There was reasonable positive correlation between elevated serum phosphatase values and presence of rickets as determined by X-ray examination.

Serum calcium was significantly lowered during active rickets in cats fed the diet with a 1:1 Ca:P ratio but not in rachitic cats receiving the diet with these minerals in the ratio of 2:0.65. Serum phosphorus and serum citric acid levels were generally lower in rachitic cats receiving the vitamin D supplement.

The ash content of fat-free femurs was found to exceed 50 per cent for all cats which received vitamin D, and also for cats which survived the entire experimental period of 21 months on the vitamin D-deficient diets. Less bone ash was found in the cats that succumbed and were rachitic, the lowest value of 36 per cent occurring in a rachitic fatality at nine months of age. Typical marked changes producing the characteristic "rachitic rosary" were observed at the costo-chondral junctions in the vitamin D-deficient cats.

Although these studies demonstrate that kittens are susceptible to rickets, it is evident that the basal rations employed did not provide uniformly desirable performance. In addition to pneumonia and purulent bron-

chitis, which was found in some animals, fatty nephrosis was observed in five cats at autopsy. All animals with this lesion succumbed during the course of the experiment and vitamin D in the diet appeared not to influence the condition.

Rickets in cats receiving the diet with a Ca:P ratio of 2:0.65 was characterized by spontaneous exacerbations and remissions as evidenced by both X-ray and serum phosphatase determinations. The authors noted that such spontaneous occurrences have not been reported in other species. Perhaps the variability in acceptance of the diet, resulting in varying alternative periods of growth depression and growth acceleration, represents a unique nutritional pattern which if, applied to other species, might also reveal exacerbations and remissions in the rachitic process. The need for further studies aimed at developing adequate experimental diets for cats is evident from these experiments.

CALCIUM METABOLISM OF DAIRY COWS

A study of the influence of the ratio of calcium to phosphorus in the diet on calcium metabolism in the dairy cow has been reported by J. R. Luick, J. M. Boda and M. Kleiber (*Am. J. Physiol.* **189,** *483 (1957)*). Their interest in this problem arose from earlier observations (Boda and Cole. *J. Dairy Sci.* **37,** *360 (1954)*) that cows pre-fed a low-calcium high-phosphorus diet were better able to maintain normal serum calcium levels during lactation than were those pre-fed high-calcium low-phosphorus diets.

The researchers used four sterile Jersey cows as the experimental animals. These were fed for 60 days or more on diets that differed appreciably in calcium and in phosphorus content. Four diets with Ca:P ratios of 1:7, 1:1, 5:1 and 6:1 were used. After the prefeeding period each cow was injected intravenously with approximately 5 millicuries of Ca^{45}. Blood samples taken at frequent intervals up to 150 days after injection were analysed for total calcium and for radioactive calcium. At the end of the experiment the cows were killed, tissue samples were taken for analysis, and bone sections were prepared. Autoradiograms of the bone sections were made in order to determine the distribution of radioactive calcium.

From the calcium and radioactivity measurements the authors calculated the size of the "mobilizable calcium pool" and the "turnover rate" of the calcium by isotope dilution. The basic assumption was that at some time after injection the specific activity of the various pools of calcium should become equilibrated. Thereafter the disappearance of Ca^{45} should become linear with time on a logarithmic plot, and should depend only upon the diluting effect of absorbed calcium. According to the authors, previous work on this subject suggested that the equilibration processes were rapid and that the skeletal calcium pool was quite small. Using this information, they calculated that the logarithmic plot should become linear within 24 hours. However, linearity was not attained in their experiment until at least 35 days after the injection. This they interpreted as indicating that the isotopic calcium becomes distributed throughout a very large pool of skeletal calcium.

After equilibration had been achieved, the authors extrapolated the linear portion of the curve back to zero time in order to obtain an estimate of the specific activity that would have been expected if the isotope had been completely mixed throughout the larger pool immediately upon injection.

With this information, and a knowledge of the amount and specific activity of the radioactive calcium that was injected, the size of the calcium pool can be calculated by isotope dilution. Also the "turnover rate" of the pool can be calculated from the slope of the curve representing disappearance of Ca^{45} after equilibration has been achieved.

Calculating from their data they obtained values indicating that the metabolizable calcium pool of the cows pre-fed the low calcium diets represented 60 per cent of the total body calcium, whereas for those pre-fed high calcium diets the comparable value was 36 per cent. Also, the calcium pool of cows pre-fed the high calcium diet turned over in about half the time required for that of cows pre-fed the low calcium diet.

The autoradiograms indicated that cows pre-fed the low calcium diet deposited much more radio-calcium in their bones than did those pre-fed the high calcium diet. There were, however, no obvious effects of the type of diet on bone structure or on the amount of mineral in the bone. A comparison of the specific activity of the serum calcium with that of the bone calcium at the end of the experiment indicated that all of the calcium of the trabecular bone was in equilibrium with the serum calcium regardless of the Ca:P ratio of the diet. Trabecular bone is known to serve as a calcium reservoir (*Nutrition Reviews* **10,** *97 (1952)*). In contrast, however, only 7 to 8 per cent of the calcium of the cortical bone of cows consuming the high calcium diets was in equilibrium with the serum calcium, whereas as much as 17 per cent of the calcium of cortical bone was in equilibrium with serum calcium when the Ca:P ratio of the diet was 1:7.

It was also observed that the serum calcium level was highest (11.4 mg. per cent) when the ratio of Ca:P in the diet was 1:7, and that the serum calcium level fell as the Ca:P ratio increased. The value for serum calcium, when the Ca:P ratio of the diet was 6:1, was 9.5 mg. per cent.

It is evident from these results that both the size of the "metabolizable calcium pool" and its "turnover rate" varied with the Ca:P ratio of the diet. However, these varied in opposite directions so that their product or the "transfer rate" remained almost unchanged. The cow fed on the diet having the lowest Ca:P ratio was in negative calcium balance, showed a reduced rate of absorption, and showed an increased rate of fecal excretion. Positive balance was achieved and absorption was improved in each case when the Ca:P ratio exceeded one.

The authors speculate that when a cow is fed on a diet with a low Ca:P ratio, the percentage of dietary calcium absorbed falls, and there is a decreased retention of body calcium. To compensate for this, and to maintain an adequate level of serum calcium, the amount of body calcium which is mobilizable increases. Since the cow is in negative calcium balance the increase in mobilizable calcium must take place at the expense of stable bone calcium, a condition which can, of course, continue for only a limited period of time.

This sequence of events provides a picture of the metabolic changes which occur when dairy cows are fed diets having low Ca:P ratios, and indicates why the feeding of a diet with a low Ca:P ratio prior to parturition prevents the development of milk fever (hypocalcemia) during lactation. This represents an interesting case of a complex metabolic adaptation which occurs in response to a dietary change. The stimulus responsible for bringing this mechanism into play is thought to be increased secretion of parathyroid hormone, which is known to affect calcium mobilization and bone resorption. Only prolonged hypersecretion of the hormone would account for the continued elevation of serum calcium level.

PYRIDOXINE, PANTOTHENIC ACID AND HEME SYNTHESIS

Anemia has been reported as one of the signs of pyridoxine (*Nutrition Reviews* **1,** *217 (1943)*; **3,** *29, 269 (1945)*; **6,** *137 (1948)*; **7,** *184 (1949)*; **8,** *15, 182 (1950)*) and pantothenic acid (*Ibid.* **4,** *91 (1946)*; **8,** *156 (1950)*) deficiencies in a number of species. The observation that there was a reduction in the level of protoporphyrin in the erythro-

cytes of swine fed on pyridoxine-deficient diets suggested that the anemia caused by this deficiency resulted from a defect in protoporphyrin synthesis (Cartright and Wintrobe, *J. Biol. Chem.* **172,** *557 (1948)*). Now that the pathway of porphyrin biosynthesis has been outlined in considerable detail (Shemin, *Fed. Proc.* **15,** *971 (1956)*; Berlin, Neuberger and Scott, *Biochem. J.* **64,** *80 (1956)*) and has been shown to depend on reactions involving amino acids and intermediates of the tricarboxylic acid cycle, a theoretical basis for the involvement of pyridoxine and pantothenic acid in porphyrin metabolism can be developed. With this background in mind, M. P. Schulman and D. A. Richert (*J. Biol. Chem.* **226,** *181 (1957)*) have studied porphyrin synthesis in ducklings fed on diets deficient in either pyridoxine or pantothenic acid.

The experimental procedure consisted primarily of incubating red cells (either whole blood or a hemolyzate prepared from washed cells) with a porphyrin precursor that contained C^{14}, isolating the heme as crystalline hemin and determining the specific activity of the isolated hemin. The ducklings from which blood was obtained were raised for up to ten days on a purified diet that was complete, or from which either pyridoxine or pantothenic acid had been omitted. Blood was drawn from the hearts of anesthetized birds and a 2 ml. aliquot was incubated at 38°C. in air for two hours with 20 micromoles of glycine-2-C^{14}, 20 micromoles of succinate-2-C^{14} or 2 micromoles of δ-aminolevulinic acid-2,3-C^{14}. After incubation hemoglobin was added as a heme carrier, the hemin was isolated, and its radioactivity was determined using a flow counter.

The first series of experiments demonstrated that a pyridoxine deficiency reduced the ability of red cells to incorporate glycine-2-C^{14} and succinate-2-C^{14} into hemin. Whole blood from ducklings that had been fed the pyridoxine-deficient diet for five days incorporated only about half as much glycine or succinate into hemin as did whole blood from control birds. The addition of pyridoxal phosphate to the blood of pyridoxine-deficient ducklings increased the incorporation of the heme precursors by about 60 per cent. Although a similar stimulation could be demonstrated in blood from ducklings that had consumed the deficient diet for ten days, it was evident that the overall heme synthesis, with or without pyridoxal phosphate, was appreciably lower. Only after ten days was there a significant decrease in the ability of the blood from pyridoxine-deficient ducklings to incorporate δ-aminolevulinic acid, and this decrease was not prevented by the addition of pyridoxal phosphate to the incubation mixture.

These results suggested that pyridoxal phosphate was required for the synthesis of δ-aminolevulinic acid from glycine and succinate. However, the results with δ-aminolevulinic acid indicated that a vitamin B_6 deficiency interfered with heme synthesis at some other point as well. This was further suggested by the results of an experiment in which δ-aminolevulinic acid injected into pyridoxine-deficient ducklings failed to decrease the severity of the resulting anemia.

In the second series of experiments, hemolyzates prepared from the blood of pantothenic acid-deficient ducklings were used. This deficiency also caused a severe depression in the incorporation of glycine and succinate into heme. This effect could be demonstrated in blood samples taken from ducklings fed the deficient diet for only 2.5 days and was more severe than that observed in the case of pyridoxine deficiency. Again there was little effect on the incorporation of δ-aminolevulinic acid into heme during the early stages, but some depression was observed by the sixth day. The addition of coenzyme A to the incubation mixtures was without effect, but when calcium pantothenate was injected into the deficient birds one hour before blood samples were drawn, the rate of incorporation of glycine into heme was restored to a normal value.

The observations on the effect of pantothenic acid on the incorporation of glycine and succinate into heme support the suggestion of Shemin and Russell (*J. Am. Chem. Soc.* **75,** *4873* (*1953*)) that succinate is converted to succinyl-coenzyme A before it reacts with glycine.

These observations of Schulman and Richert provide evidence for a specific biochemical defect which would account for reduced heme synthesis, and, therefore, anemia in animals ingesting diets deficient in either pyridoxine or pantothenic acid. The effects of a pyridoxine deficiency appear to be quite complex and will probably be more difficult to pinpoint than those of pantothenic acid deficiency. Nevertheless, these results represent a stride toward providing a sound biochemical basis for deficiency symptoms and their extension should lead eventually to the completion of an interesting chapter in the study of nutritional anemias.

COMPARISON OF HIGH-EXTRACTION AND ENRICHED FLOUR AND BREAD

It may be recalled that there was considerable concern over the adequacy of British and American diets at the beginning of World War II.

The principal direct action taken in the United States was that of continuing the use of wheat flour of low extraction (70 per cent extraction) but enriching it with certain vitamins and minerals. The British chose to elevate the extraction to 85 per cent (national flour) as a means of conserving wheat and the major nutrients of wheat in flour and bread.

H. M. Sinclair (*in Aspects of Modern Science, Ed, 1. Geikie-Cobb. London: Low* (*1951*)) has calculated that during the war, bread and flour provided the following approximate percentage requirements of nutrients in the British adult diet: calories, 35; protein, 47; carbohydrate, 48; fat, 5; iron, 71; calcium, 32; phosphorus, 52; thiamine, 65; niacin, 43; and riboflavin, 24. Sinclair further calculated the proportion of an average person's approximate daily requirement met by the 85 per cent-extraction flour and bread with added $CaCO_3$ as compared with 70 per cent extraction. These figures are recorded in Table I.

TABLE I

	85% Extraction with $CaCO_3$	70 % Extraction
Calories	27	24
Protein	37	31
Carbohydrate	37	35
Fat	4	2
Iron	46	26
Calcium	21	8
Phosphorus	33	19
Thiamine	49	13
Niacin	33	18
Riboflavin	19	7

After World War II the conference on the post-war loaf (*Ministry of Food, H.M.S.O Cmd. 6701,* (*1945*)) decided the "token nutrients" to be added to flour should be iron, thiamine, and niacin. Niacin was included, rather than riboflavin as in the U. S., because flour provided rather higher percentage requirements of niacin than riboflavin.

In a recent paper Sinclair has again reviewed the relative nutritive values of flours of high- versus low-extraction (*Royal Society of Health Journal* **77,** *234* (*1956*)). He points out that the difference in amounts of thiamine, niacin, and riboflavin found in National bread and bread of 70 per cent extraction would probably extend to other nutrients. Little is known about the human requirements for pyridoxine, pantothenic acid, biotin, folacin and vitamin B_{12}. According to Sinclair, high-extraction flour contains nearly three times as much pyridoxine, twice as much pantothenic acid and

folic acid, and nearly five times as much biotin as low-extraction flour.

It is also pointed out that high extraction flour contains 1.7 per cent ash while white flour contains only 0.37 per cent, the former containing four times as much copper and 70 times as much manganese as the latter.

Reference is made to the careful study by E. M. Widdowson and R. A. McCance in which 85 per cent extraction bread was compared with enriched white bread in a German orphanage (*Nutrition Reviews* **13,** *131 (1955)*). The children in the orphanage, who were undernourished at the start, grew rapidly on both types of flour even though the diet was very low in products derived from animal sources. Sinclair challenges the usefulness of the diets as a means of establishing the relative merits of high-extraction versus enrichment of bread. He points out that: (1) All known nutrients, except riboflavin, appear to have been present in the orphanage diet in sufficient amounts to meet requirements; (2) the experiment was of short duration; and (3) the 70 per cent extraction flour used was unusually rich in certain nutrients and in some respects more nearly resembled 80 per cent rather than 70 per cent extraction.

Of more immediate interest is Sinclair's discussion of the fat components of flours of high- versus low-extraction as this may relate to atherosclerosis in man. It is said that whole wheat flour contains about 1 g. of linoleic acid per 100 g., a level which may not be accepted by all. If, therefore, 100 ounces of flour and bread are consumed weekly, about 4 g. of linoleic acid would be consumed daily. Although the essential fatty acid requirement for man is not known, if it is assumed to be 1 per cent of the total calories, the daily requirement would be about 3.3 g. Sinclair states that white flour contains only about one-half as much linoleic acid as whole wheat flour.

Vitamin E is also mentioned; flours of 82 to 100 per cent extraction are reported to contain 5.9 mg. of tocopherols per 100 g., whereas white flour contains only 1.7 mg. Although the human requirement for vitamin E is not known, Sinclair points out that it may have an important role in protecting essential fatty acids against oxidation.

Despite Sinclair's view, the British in 1955, as a result of the action of an impartial panel, adopted the policy that a lowering of the flour extraction rate from 85 per cent to 70 per cent is very unlikely to lead to any nutritional disturbances.

DIET AND THE OVARIAN CYCLE OF THE BABOON

The hormonal changes of the menstrual cycle of women induce general metabolic changes. The rise in body temperature which occurs at the time of ovulation and persists during the luteal phase of the cycle is due largely to the action of progesterone. This physiologic change is employed in gynecologic practice to determine the time of ovulation. Cyclic changes in blood carbon dioxide tension also correlate with the menstrual cycle. A lowering of the carbon dioxide partial pressure in the alveolar air has been induced in both men and women by the administration of progesterone (I. Heerhaber, H. H. Loeschch and U. Westphal, *Arch. f. d. ges Physiol.* **250,** *42 (1948)*). Some women show evidence of edema premenstrually (G. W. Thorn, K. R. Nelson and D. W. Thorn, *Endocrinology* **22,** *155 (1938)*) and have increased intake of food and water during the premenstrual period.

The metabolic changes which have been observed in women are of mild degree compared to the pronounced changes which occur in female baboons. In this species significant alterations of plasma proteins, serum lipids, protein-bound iodine, electrolytes and blood volume occur during the

menstrual cycle. Particularly striking is the effect of ovarian function on food intake which has been observed in detail by C. Gilbert and J. Gillman (*S. Afr. J. Med. Sci.* **21,** *75* (*1956*)).

These workers have developed a diet which permits normal reproductive function in the baboon. The ration includes maize meal, skimmed milk powder, ox liver, fat, and fresh fruits and vegetables. Three female baboons were studied in metabolism cages during a period of three menstrual cycles to determine the changes in food intake during this period. The phases of the cycle are easily recognized by the massive turgescence of the sexual skin which occurs during the period of ovarian follicle development. External measurements of the degree of turgescence provide an index of estrogenic activity. The mean caloric intakes of the three baboons were not directly related to body weight. During the cycle, the caloric intake was lowest during the follicular phase and increased abruptly after ovulation. Increased food intake was sustained until two to seven days prior to the onset of menstruation. The daily administration of 5 mg. of progesterone starting in the preovulatory period induced a rise in food intake which was similar to that which occurred during the luteal phase of normal periods. It would seem likely that this hormone is responsible for the major changes in food intake.

The changes in serum protein during the cycle are particularly striking. In one chart presented, the serum protein of one baboon was about 6 g. per 100 ml. during the early ovulatory period and rose to over 9.5 g. soon after ovulation. These changes of serum protein are not believed to be due to simple alterations of water balance but may be related to sequestration of serum proteins in the area of turgescence of the genitalia and the release of these proteins after ovulation. Protein intake was actually greatest when the circulation is flooded with protein. The mechanisms involved in this peculiar reaction are still quite obscure.

As in the human being, dietary deficiencies disrupt ovarian function. The menstrual disturbances associated with malnutrition have been described in a second paper by Gillman and Gilbert (*S. Afr. med. Sci.* **21,** *89* (*1956*)). Omissions of various components of the standard diet of the baboons in the colony were examined for the effect on the menstrual cycle. With diets of maize alone, of maize, skimmed milk powder and fat, or of fruits and vegetables alone, there rapidly developed amenorrhea. Because the evidence of sex skin stimulation often persisted despite amenorrhea, the authors postulated that a failure of ovulation had resulted from an inadequate release of the pituitary luteinizing and luteotrophic hormones. Examination of the ovaries of baboons dying of malnutrition during the experiments provided support for this hypothesis. Many small and medium sized follicles were seen without corpora lutea.

In addition to the reproductive disturbances, the baboons showed other evidences of malnutrition. Weight loss was severe. On diets without liver or vegetables death occurred with gastrointestinal ulcerations and a severe interstitial nephritis.

The specific deficiencies of the experimental diets were not defined. The absence of liver from the diets containing maize alone could not be replaced with vitamins A and C, folacin, folinic acid or vitamin B_{12}. When the experimental diet was comprised of fruits and vegetables, skimmed milk powder without liver maintained body weight and reproductive function.

It is obvious from these reports that the metabolic and nutritional alterations during the menstrual cycle of the baboon are much more obvious than in humans. The ease of continuous evaluation of estrogenic function makes experimental use of this species profitable. The papers contain a great body of data recorded in detail. The clinical descriptions of the manifestations of deficiency during the dietary experiments are very clear and the demonstration of the depend-

ence of normal ovarian function on adequate nutrition is unequivocal. The disappointing feature of the study is the undefined nature of the dietary deficiencies which the experimental diets induced. An important nutritional question remains unanswered. Are the menstrual disorders the result of specific deficiencies impairing the function of the anterior pituitary or ovary, or are they the result of the general debility of malnutrition? This question can be answered with the use of purified and chemically defined rations.

VITAMINS FROM RHODOTORULA YEAST

Some years ago it was discovered that when the yeast, *Rhodotorula gracilis*, was grown in a nutrient solution low in nitrogen, but containing an excess of sugar, considerable amounts of fat were produced (L. Enebo, L. G. Anderson and H. Lundin, *Arch. Biochem.* **11,** *383 (1946)*). Under these conditions the fat content amounted to as much as 60 per cent of the total dry material, and the protein was as low as 12 per cent of the dry weight. On the other hand, if the yeast was cultivated in an excess of nitrogen, an organism containing 50 per cent protein and only 10 per cent fat was obtained.

Comparative studies of the protein-rich and protein-poor organisms were carried out by N. Nielsen, E. Sandegren and L. Ljungdahl (*Nature* **164,** *1055 (1949)*). The amino acid content was found to be somewhat different in the two types. The protein-poor yeast had a relatively high content of glutamic acid and alanine as compared with the protein-rich yeast. β-alanine, which is a component of pantothenic acid (and, thus, coenzyme A) was present in the protein-rich organisms, but not in the protein-poor ones. The protein-poor yeast was also found to be very low in cystine and methionine.

Since these differences in metabolism could very obviously lead to important variations in the vitamin-forming potentialities of this yeast, Nielsen and G. Schneider (*Acta chem. Scandinav.* **11,** *685 (1957)*) have recently investigated the ability of these two types of yeast to form riboflavin, pantothenic acid, niacin and pyridoxine. Five experiments with a nitrogen-rich, and five with a nitrogen-poor, nutrient solution were carried out. In four experiments (two on each type) ammonium sulfate was the source of nitrogen while in the others, asparagine was used.

A large difference in pH in the fermenting medium resulted from the use of ammonium sulfate and asparagine. Whereas the pH was constant at approximately 5 during fermentation with asparagine, the final pH in the cases where ammonium sulfate was used was about 2. The acidity of the latter was due to the fact that, as the ammonium ions were taken up, the solution became more acid. This acidity was thought by the authors to be the reason for a somewhat slower rate of growth of the yeast when ammonium sulfate was the source of nitrogen.

It was found that relatively little fat was formed when ammonium sulfate was the nitrogen source. This was again probably the result of the acidity of the nutrient solution.

The vitamins riboflavin, pantothenic acid, niacin and pyridoxine were determined both in the cell-free nutrient solution and in the yeast cells. It was found that a larger quantity of the vitamins was present in the nutrient solution (50 to 80 per cent). The amount of pantothenic acid present was correlated with the crude protein content of the yeast. The reason for this may be that β-alanine is not formed rapidly enough in nutrients deficient in nitrogen. Differences in the amounts of the other three vitamins in protein-rich and protein-poor yeast were not so striking, although the amounts were some-

what smaller for the protein-poor yeast. If calculated on the basis of grams of protein, the amounts of vitamins in protein-poor yeast are relatively higher, with the exception of pantothenic acid. This was especially apparent in the case of niacin which seemed to be produced in higher amounts when the yeast was cultivated in the ammonium sulfate medium, a result which was probably related to the low pH of the nutrient solution.

In earlier investigations it was found that addition of thiamine and pantothenic acid stimulated the growth of this yeast but that niacin, riboflavin or pyridoxine had no effect. This of course, agrees with the above finding that pantothenic acid, under conditions poor in nitrogen was formed only in small amounts.

If these findings may be applied to the commercial preparation of yeasts for animal, or possibly even human, use, it would appear that yeast grown on ammonium sulfate would be at the same time both more economical and nutritionally desirable. The higher yields of niacin and comparable yields of other vitamins combined with less fat and more protein suggest the means of production of a strikingly nutritious product. Culture media should also be gleaned for vitamins since the higher proportion of these are apparently lost from the yeast bulk.

CALCIUM METABOLISM IN RATS

In recent years evidence has accumulated that vitamin D, in addition to its effect on the absorption of calcium, has a local effect in the bones and also a systemic effect. The biochemistry and physiology of vitamin D was recently reviewed (R. Nicolaysen and N. Eeg-Larsen, *Vitamins and Hormones* **11,** *29* (*1953*)) and the mode of action of vitamin D was also re-examined. Some of the additional investigations which remain to be carried out include long term calcium metabolism experiments. Since rats have been extensively used in vitamin D studies, Nicolaysen and co-workers have focused attention on long term studies on this animal. The following experiments have been carried out by the group: (1) Long term studies in rats given optimal diets with and without vitamin D (R. Haavaldsen and Nicolaysen, *Acta physiol. Scandinav.* **36,** *107* (*1956*)); (2) The interaction of vitamin D and an endogenous factor affecting absorption of calcium (Haavaldsen, K. M. Egnund and Nicolaysen, *Ibid.* **36,** *108* (*1956*)); (3) Adaptation to low calcium intake by old rats (Nicolaysen, *Ibid.* **36,** *114* (*1956*)).

In the first experiment, only male littermates were used. The composition of the diet was as follows (in per cent): casein, 10; egg albumin, 3; brewers' yeast, 3; arachis oil, 5; wheat flour, 76; vitamin B_{12} mixture, 0.5; NaCl, 1; KH_2PO_4, 0.8; and $CaCO_3$, 0.5. Vitamin D (70 I.U.) was given weekly to appropriate groups.

It was found that the effect of vitamin D on the absorption of calcium was marked only during the first 15 weeks. Thereafter the two groups (vitamin deficient and vitamin supplemented) absorbed practically the same amounts of calcium. In spite of a remaining deficiency in total body calcium, the vitamin D-free rats did not absorb enough additional calcium so as to make this up. Rates of growth were high in both groups, but the vitamin D-deficient rats lagged slightly behind the others, and when the experiment was terminated they weighed significantly less than the rats receiving vitamin D. The bones were more densely mineralized when vitamin D was administered and thus contained less water and matrix than did comparable bones in vitamin D-deficient rats of the same age.

In the second experiment, five different series were studied in an effort to elucidate

the interaction between vitamin D and a possible endogenous factor concerned with the absorption of calcium. In the first series, the dietary calcium was set at 0.25 per cent. No vitamin D was given until the rats were six months of age. At this time vitamin D resulted in increased absorption and retention of calcium. As saturation of the bones with calcium increased, there was a decrease in absorption. This latter phenomenon has suggested to the authors that something other than vitamin D *per se* is involved in the absorption of calcium from the gut.

In the second and third series, the rats were pretreated for 35 weeks on a vitamin D-free, 0.5 per cent Ca and 0.5 per cent P diet. Increased absorption of Ca resulted immediately upon administration of vitamin D and was very striking in magnitude.

Series IV and V featured the addition of 0.5 per cent sodium oxalate to the diet. In this case the calcium was actually resorbed from the bone and other tissues. Consequently the animals were in a negative calcium balance. However, when 10 I.U. of vitamin D were administered to the rats in series IV, a large increase in absorption of Ca was noted. Moreover, the uniform application of vitamin D in the V series resulted in some degree of adaptation of Ca absorption to meet the increased Ca deficiency imposed by 0.5 per cent Na oxalate.

In the final experiment, old rats (21 months) were investigated with respect to calcium absorption on low calcium diets. Seventy I.U. of vitamin D were given weekly. On this regimen, the rats maintained their calcium balance remarkably well.

These studies point up the roles which vitamin D may play in calcium absorption in adult and advanced life as well as during the growth of the skeleton. It would appear that a certain percentage of a calcium "deficiency" in the diet may be compensated by an adequate supplement of vitamin D. The vitamin appears to act in conjunction with an endogenous factor in increasing the efficiency of uptake of calcium from the gut at least in these cases where calcium is especially required by the skeleton. The endogenous factor seems to be increased in concentration by calcium deficiency but is incapable of increasing absorption in the absence of vitamin D.

VITAMIN E AND SELENIUM, PART I

An interesting story is developing, relating selenium to vitamin E (and probably to cystine) metabolism and establishing this metal as an essential trace element in at least some species. The findings grow out of the study of vitamin E in poultry nutrition on the one hand, and efforts to identify K. Schwarz's "factor 3" on the other. The long confusion over th. ıation between dietary liver necrosis and fatty liver-cirrhosis complex was resolved after F. S. Daft, W. H. Sebrell and R. D. Lillie clearly showed that these two were separate experimental entities (*Fed. Proc.* **1,** *188* (*1942*); *Proc. Soc. Exp. Biol. Med.* **50,** *1* (*1942*)). This has permitted work on dietary liver necrosis to advance considerably in the intervening years (*Nutrition Reviews* **13,** *49* (*1955*)).

K. Schwarz observed (*Proc. Soc. Exp. Biol. Med.* **77,** *818* (*1951*); *Ibid.* **78,** *852* (*1951*); *Nutrition Reviews* **10,** *288* (*1952*)) that rats, fed a diet whose protein was supplied as Torula yeast, developed necrosis of the liver. This could be prevented by cystine, vitamin E, or an unknown third substance, present in vitamin-free casein and in brewers' yeast, which he called "factor 3". This factor has been the object of research for several years, culminating in its identification as selenium by K. Schwarz and C. M. Foltz (see below). What the relation, if any, may be between cystine,

vitamin E and selenium, is not yet clear, but there evidently is some, at least between the last two.

Studies on vitamin E deficiencies have been complicated by the presence of trace amounts of the vitamin in the basal rations used. Since this compound is an antioxidant, the diets have usually been treated with ferric chloride, or a vitamin E-oxidizing fat has been added, *e.g.* lard or cod liver oil. Thus, as investigators are well aware, vitamin E deficiency is conditioned by factors quite unrelated to the amount of the vitamin in the diet (H. Dam, *J. Nutrition* **27,** *193 (1944)*).

Dam's extensive studies have shown that highly unsaturated fats are necessary for symptoms to result from diets low in vitamin E and that antioxidants, including methylene blue, spare the vitamin (*Nutrition Reviews* **10,** *149 (1952)*). Others have confirmed and extended these observations (*Ibid.* **14,** *141 (1956)*). The unexplained lack of correlation between the incidence of the two recognized vitamin E-deficiency syndromes of the chick, exudative diathesis and encephalomalacia, points up the complexity of this problem. Dam clarified this partially when he showed that the fatty acids from cod liver oil, lard and linseed oil favored the production of exudative diathesis, while those from pig liver tended to produce encephalomalacia. He found a number of lipids and lipotropic agents which affected the development of one or the other, or both, syndromes.

For these reasons M. L. Scott, *et al.* (*J. Nutrition* **56,** *387 (1955)*) undertook to develop a basal chick diet so free of vitamin E that deficiency symptoms would not be enhanced or suppressed by pro-oxidants or antioxidants. They thought that Schwarz's Torula yeast diet for rats, modified to meet the requirements of the chick, might fulfill these requirements and permit research on the uncomplicated role of vitamin E in poultry nutrition. In addition, Scott had observed that vitamin E was concerned in the prevention of the "enlarged hock disorder" of turkeys and that dried brewers' yeast, which contains none of this vitamin, appeared to spare the need for it in this condition.

Scott, *et al.*, showed that their diet (which contained 58.5 per cent Torula yeast and 5 per cent vitamin E-free stripped lard) produced symptoms unaffected by the addition of pro-oxidants or antioxidants (*loc. cit.*; *Nutrition Reviews* **14,** *141 (1956)*). The exudative diathesis developed by the third week and was fatal in less than four weeks. Recovery occurred within 24 to 48 hours after a single oral dose of 10 mg. of D-α-tocopheryl acetate. No liver necrosis was found, even terminally. Interestingly, though many chicks showed slight symptoms and lesions of encephalomalacia, none was severely affected. Brewers' yeast was as effective as vitamin E in preventing symptoms of the vitamin deficiency. The agent was not removed from the yeast by hexane extraction, and thus was clearly different from the fat-soluble vitamin E. The course of the deficiency disease was unaltered by omission of lard from the basal diet. Deficient birds were found to have a microcytic anemia with far fewer reticulocytes than those receiving vitamin E (15 compared to 85 per cent). The unknown factor in brewers' yeast prevented anemia as well as the other symptoms.

Thus there are at least two conditions prevented by either vitamin E or the "factor 3" of Schwarz: dietary liver necrosis in the rat and exudative diathesis (and "enlarged hock disorder") in the chick. Whether "factor 3" also prevents other vitamin E deficiency lesions, such as encephalomalacia in chicks or skeletal muscle necrosis and fetal resorption in rats, is not clear at present.

As a result of the observations of Schwarz and of Scott, *et al.*, E. L. Patterson, R. Milstrey and E. L. R. Stokstad (*Proc. Soc. Exp. Biol. Med.* **95,** *617 (1957)*) investigated the identity of the factor which

prevents exudative diathesis in chicks. They found that vitamin E-free casein and several defatted pork tissues were preventive, and they undertook to isolate the material by a variety of laboratory methods. The activity was adsorbed on sodium cycle and hydrogen cycle resins, suggesting that it was amphoteric. Kidney ash made alkaline before burning was active; ash of acid hydrolyzed kidney was inactive. The authors inferred that the activity in kidney might be due to an element which formed a volatile acid.

Since arsenic, selenium and tellurium form volatile acids, the authors investigated the possibility that one of these elements might prevent exudative diathesis in chicks. They used a basal vitamin E-deficient diet, containing 40 per cent Torula yeast and 5 per cent vitamin E-free lard. Day-old White Rock chicks were put on the diet for six to 13 days before being given the test diets. Signs of exudative diathesis were first observed on the eighteenth day. Tocopheryl acetate was protective, as was casein extracted with boiling alcohol. A simulating amino acid mixture was inactive. Several defatted pork tissues were tested and kidney and pancreas were found the most active. A sulfuric acid hydrolysate of kidney was selected for fractionation work; 30 g. of this per kg. diet were required for protection. Brewers' yeast was apparently not tested. The hydrolyzed kidney contained a selenium concentration of 3.5 parts per million (p.p.m.); 20 per cent was recovered in the alkaline ash and seven per cent in the acid ash. Selenium (as selenite) was completely protective at 0.3 p.p.m. Tellurium (as tellurite) was inactive at 3 p.p.m., as was arsenic (as equal mixture of arsenate and arsenite) at 10 p.p.m.

Patterson's group concludes that "selenium can replace vitamin E for prevention of exudative diathesis on chicks on a Torula yeast diet." The Torula yeast contained less than 0.1 p.p.m. of selenium, contributing under 0.04 p.p.m. in the basal diet. Therefore, although Patterson's group could not state that vitamin E completely eliminated the need for selenium, the requirement could not be over 0.04 p.p.m., assuming that this was all that the diet contained.

K. Schwarz and C. M. Foltz (*J. Am. Chem. Soc.* **79,** *3292 (1957)*; *Nutrition Reviews* **15,** *319 (1957)*) have published an article entitled "Selenium as an integral part of factor 3 against dietary necrotic liver degeneration", which appeared after that of Patterson's group had been submitted. Schwarz and his colleagues have sought to identify this factor since 1951. They have prepared various concentrates which are protective not only against dietary liver necrosis in the rat, but against "multiple necrotic degeneration (heart, liver, kidney and muscle necrosis) in the mouse" and against exudative diathesis in the chick. Schwarz's group produced these lesions by diets low in cystine and also deficient in vitamin E and "factor 3". They state that each substance alone is protective.

In the course of fractionation of "factor 3", the authors found that activity resided in at least two chemically related substances, which they called "alpha-" and "beta-factor 3". The former was water soluble, anionic, sensitive to reducing agents and its activity was destroyed by dry ashing. Purified fractions developed a garlic-like odor upon addition of alkali, suggesting the presence of selenium. Various fractions from a hydrolysate of dried, defatted kidney powder were analyzed for selenium and a rough correlation was found with "factor 3" activity as determined by bio-assay. When inorganic selenium salts were tested they proved "remarkably effective" in protecting against necrotic liver degeneration; as little as 13.33 μg. of sodium selenite per 100 g. diet were completely protective. This amounted to 4 μg. selenium per 100 g. diet, or about 0.25 μg. per rat per day. Two thirds of this dose, given subcutaneously, was protective. Potassium selenate was less effective and potassium tellurite and sodium arsenate (which antagonizes selenium

poisoning in various species) had no activity. The authors found that selenium, when given as sodium selenite, was 500 times as active as vitamin E and 250,000 (*sic*) as active as L-cystine.

Schwarz and Foltz point out that selenium is regularly present in tissues of higher animals and conclude that it is an essential trace element. They state that "a characteristic defect, respiratory decline, is present in oxidative metabolism of liver slices from animals on necrogenic diets several weeks before necrosis occurs" (S. S. Chernick, J. G. Moe, G. P. Rodman and K. Schwarz, *J. Biol. Chem.* **217,** *829* (*1955*)) and suggest that selenium may take part in oxidation-reduction reactions (see part II of this review, which will appear next month).

EFFECT OF GROWTH HORMONE ON FAT SYNTHESIS

It has been demonstrated in many types of experiments that pituitary growth hormone exerts its protein sparing action, at least in part, by effecting increased fat oxidation (*Nutrition Reviews* **12,** *86* (*1954*)). This effect is particularly striking when animals on a restricted diet are treated with growth hormone (A. L. Greenbaum, *Biochem. J.* **54,** *400* (*1953*)). The marked decrease in body fat which results has been thought to be too great to be accounted for by the increased fat catabolism in the liver or the extra-hepatic tissues. In agreement with these observations several investigations have appeared to show a decreased fat synthesis under the influence of growth hormone. For instance, R. O. Brady, F. D. W. Lukens and S. Gurin (*J. Biol. Chem.* **193,** *459* (*1951*)) have reported a profound inhibition of fatty acid synthesis by liver slices of Houssay cats (hypophysectomized, pancreatectomized) injected with growth hormone. A. Allen, G. Medes and S. Weinhouse (*Ibid.* **221,** *333* (*1956*)), however, have failed to confirm these findings.

Greenbaum and R. F. Glascock (*Biochem. J.* **67,** *360* (*1957*)) have carried out experiments designed to answer the question of the effect of growth hormone on fatty acid synthesis. Since one of the authors, Greenbaum, had previously found that growth hormone exerts its greatest effect in changing the metabolic patterns during the first few hours after administration (Greenbaum, *Ibid.* **63,** *159* (*1956*)), fatty acid synthesis was studied at six and 12 hours.

Liver slices were prepared from rats which had been injected with 1 mg. of growth hormone and were incubated in the appropriate medium with 1-C^{14} acetate, 1-C^{14} pyruvate and 2-C^{14} pyruvate. The activity of carbon dioxide, cholesterol, phospholipids and fatty acids was determined after two hours incubation.

No differences were observed in the activity of carbon dioxide formed by liver slices of controls and treated animals from any of the substrates. The 1-C^{14} pyruvate did not contribute significant activity to any of the lipid fractions, but the 2-C^{14} pyruvate and the 1-C^{14} acetate gave similar labeling of all three fractions. With these two substrates, inhibition of fatty acid labeling, amounting to about 60 per cent at six hours and 15 to 20 per cent at 12 hours, was observed in the livers of the hormone-treated rats. In the case of phospholipid, similar but less marked inhibition occurred. A transient stimulation of cholesterol labeling was observed at six hours, but at 12 hours the activities of control and experimental cholesterol were approximately equal.

The authors are of the opinion that the reported experiments prove that a reduction in the actual rate of fatty acid synthesis results from the action of growth hormone whether direct or indirect, and not from a dilution of active fatty acids by mobilization of inactive fat from the depots. They have no explanation for the effect on cholesterol synthesis but point out that the same sort of relationship was found by S. Hotta and I. L.

Chaikoff (*J. Biol. Chem.* **198,** *895* (*1952*)) for livers from diabetic rats. A. L. Greenbaum (*loc. cit.*) had previously shown that at six hours after injection of growth hormone, an insulin effect on fatty acid metabolism was evident. Thus it appears that, although the metabolic changes effected by growth hormone are actually present at a very early time after administration, the true effect does not become manifest until after about six hours because of an early release of insulin with its characteristic action.

DIETARY CALCIUM AND RADIOSTRONTIUM RETENTION

The problem of radioactive fallout has come under close scrutiny in recent years. Following a nuclear explosion, either from a bomb or as a result of peaceful application of atomic energy, radioactive materials are liberated. These may fall upon the soil and foliage in the immediate vicinity or may be carried into the atmosphere, depending upon the nature of the explosion. The material carried into the atmosphere and stratosphere eventually falls to the earth after time periods varying from days to ten years.

Although many radioactive materials may be formed under these circumstances most have short half-lives and present no long range health hazard. It is generally agreed that the greatest hazard in radioactive fallout is from strontium^{90} (T. H. Maher, *New Engl. J. Med.* **252,** *740* (*1955*); W. F. Libby, *Proc. Nat. Acad. Sci., U. S. A.* **42,** *365* (*1956*)). This isotope has a half-life of approximately 20 years, and, due to its chemical similarity to calcium, it will appear in biological materials in all positions normally occupied by calcium. Strontium^{90} falls upon the soil and foliage and eventually appears in all the biological calcium reservoirs including human and animal skeletons, milk, and vegetables, and in the upper few inches of soil (C. L. Comar, R. S. Russell and R. H. Wasserman, *Science* **126,** *485* (*1957*)). As a result of testing atomic weapons, strontium^{90} is accumulating, and recent studies have disclosed the presence of the isotope in human bones taken at autopsies from widely separated regions of the world (W. H. Langham and E. C. Anderson, *Ibid.* **126,** *205* (*1957*); J. L. Kulp, W. R. Ecklemann, and A. R. Schulert, *Ibid.* **125,** *219* (*1957*)). As would be expected, more of the radiostrontium is present in the bones of children than in adults.

The possible health hazard of strontium^{90} has been discussed at some length and calculations as to the effect on the incidence of leukemia (E. B. Lewis, *Science* **125,** *965* (*1957*)) and osteosarcoma (H. B. Newcombe, *Ibid.* **126,** *549* (*1957*)) have been reported. Although the present levels of strontium^{90} may be considered safe, any considerable increase in nuclear explosions might be expected to pose a real health problem.

The chemical similarity of strontium and calcium introduces a nutritional aspect of the fallout problem. Since these two elements compete with each other in metabolic reactions, the possibility of reducing radiostrontium assimilation by increasing the dietary calcium intake presents itself. This possibility has been explored by R. H. Wasserman and C. L. Comar (*Science* **126,** *1180* (*1957*)). Groups of young rats were given a diet of corn, casein, brewers' yeast, cottonseed oil, salts and the usual vitamins. Three different levels of dietary calcium were fed; 0.5, 1.0 and 2.0 per cent. The calcium-phosphorus ratio was adjusted to approximately two to one for all dietary levels. These diets were fed for 15 days in one series, and for 45 days in another. During the last seven days of each experiment the drinking water was fortified with radiostrontium (strontium^{85}). At the termination of the experiments the per cent of the ingested radiostrontium retained in the carcass was determined. In the short term experiment, animals fed 2.0 per cent calcium retained only one-third as much radio-

strontium as did animals fed 0.5 per cent calcium. In the second series (45-day feeding period), increasing the dietary calcium from 0.5 to 2.0 per cent reduced carcass radiostrontium from 17.7 per cent of the ingested dose to 3.8 per cent.

These findings demonstrate clearly that increasing the dietary calcium intake will reduce the assimilation of radiostrontium. The practical implications are obvious. In areas where there is exposure to large quantities of strontium^{90}, it may be advisable to recommend increases in dietary calcium. Such calcium should be uncontaminated with strontium^{90} and could be obtained from deep mineral deposits.

LIPOTROPIC FACTORS AND SERUM PHOSPHOLIPIDS

In spite of the large number of studies on factors that influence the deposition of liver fat, the mechanism of action of lipotropic substances remains unknown. The early discovery that choline, a component of lecithin, was a lipotropic factor suggested that its activity depended upon its incorporation into phospholipids (*Nutrition Reviews* **2,** *275* (*1944*)). The possibility that phospholipids might play a role in fat transport has been discussed from time to time (*Ibid.* **4,** *167* (*1946*)) but evidence indicating that fat transport did not depend upon the incorporation of fatty acids into phospholipids (*Ibid.* **9,** *211* (*1951*)) detracted from the hypothesis that fatty livers resulted from a failure in fat transport mechanisms.

Since it now appears that lipoproteins which contain phospholipids may be involved in the transport of neutral fat in the body, interest in the relationship between lipotropic activity and fat transport has been renewed. Actually it is surprising how little evidence there is on the influence of choline and protein, both of which exhibit lipotropic activity (*Ibid.* **12,** *22*, *151* (*1954*); **13,** *217* (*1955*)), on the level of serum phospholipids.

It is therefore of intestest that B. Rosenfeld and J. M. Lang (*Canad. J. Biochem. Physiol.* **35,** *845* (*1957*)) have reported the results of a study of the influence of choline and of dietary protein on the level of serum phospholipids in the rat.

The experimental animals were mature rats. They were fed on purified diets containing sucrose, adequate quantities of minerals, and vitamins, except for choline. The dietary fat consisted either of 2 per cent beef fat or 9 per cent hydrogenated coconut oil; the protein consisted of 6 per cent casein, 5 per cent of a purified soybean protein, and 5 per cent of gelatin supplemented with 0.1 per cent of cystine. In the experiments in which the effect of dietary protein was studied three levels of protein, 5, 9 and 19 per cent were used; each diet contained 5 per cent of casein, higher levels of protein being made up from equal parts of the proteins of peanut meal and soybeans. The diets were fed for various lengths of time up to 24 days. At the end of the experiment each rat was anesthetized, a sample of blood was taken from the carotid artery for serum phospholipid estimation, and the liver was removed and analyzed for total lipids and phospholipids.

The initial experiment consisted of a comparison of the effects of different levels of protein with, and without, choline in the diet. When the diet contained choline and the highest level of protein, the serum phospholipid value was about 200 mg. per cent. This fell to approximately 160 mg. per cent when the protein level was decreased to 9 or 5 per cent, and to between 125 and 140 mg. per cent when choline was omitted from the diet. The level of dietary protein had no significant effect on the serum phospholipid concentration in the absence of dietary choline.

In order to determine how quickly serum phospholipid values fell when choline was omitted from the diet, a time study was

made. Within three days after the omission of choline the serum phospholipid values of rats receiving the basal diet (15 per cent protein) dropped to about 120 mg. per cent and remained unchanged for a further 21 days. The fall in serum phospholipid concentration was evident before any increase in the level of liver fat could be detected. Also fasting for 16 hours did not alter the low serum phospholipid level of rats that had been fed on the choline-deficient diet, whereas 16 hours of fasting did cause a substantial decrease in the serum phospholipid values of rats previously consuming a diet containing choline. The provision of choline in the drinking water did not alter the effect of fasting.

Finally, as has been previously shown, the level of phospholipid in the liver was unaffected by the omission of choline from the diet. It was substantially increased, however, regardless of the presence or absence of dietary choline, when the level of dietary protein was increased. This effect was most noticeable when the diets contained 19 per cent of protein.

It is apparent that the level of serum phospholipids, like the level of liver fat, depends upon the amount of protein and the amount of choline in the diet. From the results of the time study it was evident that a decrease in serum phospholipids preceded the accumulation of liver fat when choline was omitted from the diet. Unfortunately none of the groups receiving choline, even with the lowest level of protein, developed fatty livers; nor did their serum phospholipid levels fall to a value as low as that observed when choline was lacking. Therefore, it was not possible to determine whether a similar relationship between liver fat deposition and serum phospholipid level existed when dietary protein was low, nor could the effects of choline and protein be compared under conditions which caused liver fat to accumulate. Nevertheless these observations are suggestive, and it would appear that further investigation of the relationship between liver lipid accumulation and blood lipid level may add appreciably to our knowledge of the role of lipotropic factors.

CYTOCHROME C LEVELS IN IRON DEFICIENCY WITHOUT ANEMIA

E. Beutler (*Am. J. Med. Sci.* **234,** *517 (1957)*) points out that iron deficiency anemia is a common clinical disorder, and that symptoms of fatigue, headache, dyspnea and paresthesias are usually ascribed to the hemoglobin deficiency without experimental evidence that this is so. Not only is hemoglobin the most easily measured iron compound in the body, but also it has been generally accepted that the body guards its iron enzymes "tenaciously" in iron deficiency. Beutler could find virtually no satisfactory proof of this statement. He was further impressed by the symptomatic improvement found in iron-deficient patients given iron, even before the hemoglobin increased; others have made the same observation. He was also struck by the lack of correlation between severity of anemia and of symptoms in iron-deficient patients and pointed out that the objective changes in nails, mouth and esophagus were not explainable on the basis of anemia and, indeed, might exist without anemia.

Not only has work on iron enzymes to date failed to show any clear changes in relation to iron-deficiency anemia, but even the changes in so abundant a compound as myoglobin have been the subject of inadequate study and conflicting conclusions. For these reasons Beutler is studying the changes in iron-containing enzymes produced by iron deficiency. His first report concerns cytochrome C. Four-week-old Sprague-Dawley rats were given an iron-deficient diet (containing 1 to 2 mg. of iron per 100 g.) or an identical diet plus ferrous sulfate (containing 21 to 22 mg. of iron per

100 g.). Blood hemoglobin concentrations were determined spectrophotometrically and calibrated pipettes were used for hemoglobin and red cell counts. The cytochrome C concentration in liver and kidneys was measured by a modified Potter and Dubois method. In the first experiment there were three rats on each diet. The two groups gained weight equally, but the blood hemoglobin concentration was markedly lower in the rats given the iron-deficient diet at three and one half weeks and minimally lower at eight weeks. At this time the animals were sacrificed. A marked decrease in cytochrome C concentration was found in liver and kidneys; in each organ the iron-deficient rats had about one third as much enzyme concentration as those fed the iron-containing diet, although the blood hemoglobin concentrations showed a minimal difference.

In a second similar experiment the rats of both groups were, in addition, bled periodically. Again, the weight gain of the two groups of three rats was similar. Although anemia developed at four weeks on the iron-deficient diet, there was none at the next bleeding (11 weeks) and later bleedings at 13, 21½ and 24 weeks were such that both groups maintained hemoglobin levels near 15 g. per cent without significant difference (there was a slight decrease at 26 weeks). Again a striking decrease in liver cytochrome C concentration was found in the iron-deficient animals (1.7 to 2.8×10^{-3} micromoles per g. compared with 7.8 to 10.6 in the rats on the normal diet). Since the livers were smaller in the iron-deficient group, the discrepancy in total liver cytochrome C was even greater. The kidney cytochrome C concentration in the deficient animals was only slightly less than in the normal rats (16.3 to 21.3 compared with 23.2 to 25.2×10^{-3} micromoles per g.). Again, the discrepancy was greater when total amounts of cytochrome C were compared.

Beutler's experiments are simple and clear. He has shown, apparently conclusively, on the basis of 12 rats, that not all iron-containing enzymes are "tenaciously protected by the iron deficient animal." Not only is cytochrome C more easily depleted than hemoglobin, but the author believes that "when only a small amount of iron is available, the hemoglobin synthesizing mechanism is more successful in competing for it than is the cytochrome C synthesizing mechanism." Other factors may well be at work here and it would be a whole project in itself to test this statement. Not only may the synthesis and turnover rates be very different, but the latter are subject to quite different determinants. Thus, red cell destruction might be very appreciably diminished in rats on the iron deficient diets. Beutler does not discuss the failure of the iron-deficient rats to show anemia, from 11 to 24 weeks, even with three small bleedings of about 2 ml. each, or their higher red cell counts (7.15 to 7.37 million per cmm. vs. 6.02 to 6.75).

Beutler's diet is stated to be "a modification" of diet 4c of R. W. Wissler, R. L. Woolridge, C. H. Steffee, Jr., and P. R. Cannon (*J. Immunol.* **52,** *267 (1946)*). This was a purified ration containing liver concentrate, six of the B vitamins, and oleum percomorphum. The original formula contains copper, but has no brewers' yeast. What Beutler's modification is, we are, unfortunately, not told. But, assuming that the diet used was deficient in no respect other than iron, and that it contained the stated 1 to 2 mg. iron per 100 g., the rats (weighing 270 g. at the end of the experiment) might consume as much as 15 g. of diet per day, containing 0.15 to 0.30 mg. of iron. Various workers have shown that 0.5 or even 0.25 mg. iron per day is sufficient for optimum hemoglobin regeneration in anemic rats, in the presence of adequate copper and other nutrients. The iron deficiency in the present experiments was therefore marginal or absent in terms of hemoglobin production, and the author has selected a level of iron intake probably ideal to test his hypothesis. It is interesting that the 0.25 mg. per day needed for optimal hemoglobin regeneration in anemic rats, which is presumably more than needed to

maintain hemoglobin levels in the non-anemic rat, is considerably less than the amount needed for reproduction and lactation. Thus we have previous support for the idea that iron sufficient for normal hemoglobin levels is not sufficient for all physiologic needs.

Beutler's work raises a number of other interesting points which are well covered in his discussion. His original reason for undertaking these studies was to verify his suspicion that the symptoms found with anemia might not all be caused by hemoglobin deficiency. The claim that there exist iron-deficient patients without anemia has usually been a loose one based on no more than a clinical impression that some tired women feel better after iron administration. Controlled studies have not been possible for lack of an objective measure. Beutler points out that oral iron tolerance curves have been used by some authors, but are really not acceptable as such a measure. Unpublished studies of the author and collaborators are mentioned, showing depleted bone marrow iron stores in a high proportion of abnormally tired women with normal circulating hemoglobin concentrations. Beutler makes no special claim that cytochrome C is the best measure of iron deficiency; he chose it because of its known importance and the fact that it can be measured independently of any blood remaining in tissues.

It is the author's plan to pursue similar studies on other iron-containing enzymes. Some day it may be possible to make a table comparing hemoglobin, myoglobin, ferritin, plasma siderophilin, cytochrome C and other iron-containing enzymes such as catalase and peroxidase. No doubt important similarities and differences among their avidities for iron will emerge in relation to dietary iron deficiency. When these substances are measured in the major varieties of anemia and hemochromatosis, we can imagine how the findings will enable us more accurately to infer the role of each of these iron-containing substances in relation to cell metabolism and hemoglobin formation.

Beutler has opened up a whole new vista of objective clinical experimentation, for we shall have in cytochrome C (or some other cellular iron component) a measure of iron deficiency to test the clinical impressions physicians may get of symptoms of iron deficiency unassociated with anemia. One must agree with the author's conclusion that, in iron deficiency, not only the blood but also the tissues deserve consideration.

In summary, cytochrome C levels are reduced in iron-deficient rats without anemia. The concept of iron deficiency without anemia has been established objectively. Some of the symptoms in anemia may be due to decreased cellular enzymes rather than to low hemoglobin levels.

ROLE OF GLUTAMINE IN TISSUE CULTURE

Although glutamine is not a dietary essential for mammalian species, it is present in relatively high concentrations in mammalian blood (Meister, *Physiol. Rev.* **36,** *103 (1956)*) and is essential for the growth of a number of cell lines in tissue culture (H. Eagle, *et al., J. Biol. Chem.* **218,** *607 (1956)*). In order to learn more about the metabolic role of this compound L. Levinton, H. Eagle and K. A. Piez (*Ibid.* **227,** *929 (1957)*) have performed a number of experiments in which they measured the extent of incorporation of the carbon and nitrogen of isotopically labeled glutamine into certain specific amino acids and the proteins of a human carcinoma cell (HeLa strain) grown in tissue culture.

Glutamine containing unlabeled N or N^{15} in the amide group was synthesized from glutamic acid uniformly labeled with C^{14}. The cells were cultured in a highly purified medium supplemented with dialyzed human serum. During the growing phase, medium

containing labeled glutamine was added to the culture, and after 48 hours of further growth in this medium the cells were washed and harvested. After harvesting, the cell proteins were isolated using a trichloracetic acid fractionation procedure. A portion of the protein was hydrolysed with hydrochloric acid, the amino acids were separated by column chromatography, the quantities were estimated by a colorimetric ninhydrin procedure and their C^{14} content was determined by measuring the radioactivity of the fractions. Glutamine and asparagine, which are converted to glutamic acid and aspartic acid during acid hydrolysis, were released unchanged from a portion of the protein by enzymatic hydrolysis. Glutamic and aspartic acids and their corresponding amides were then isolated from a dialysate of the enzymatic digest and analysed for C^{14}. The N^{15} content of the ammonia obtained from the amide groups of glutamine and asparagine was measured in a mass spectrometer.

An interesting result of this study, particularly in view of the subsequent findings, was that glutamine was rapidly broken down to ammonia and glutamic acid by the cell culture. Analysis of the amino acids isolated from the cells after incubation in a medium containing uniformly C^{14}-labeled glutamine and only the essential amino acids revealed that carbon from glutamine was present in glutamic and aspartic acids, and in the proline isolated from the cell proteins. A small amount of C^{14} was also detected in the alanine, glycine and serine. Incorporation into the latter three amino acids was abolished if the medium was supplemented with unlabeled non-essential amino acids.

Despite the fact that glutamine was rapidly broken down by the cell culture, the ammonia released was not incorporated to a significant extent into the amino acids obtained from the acid hydrolysate of the cell proteins. This observation indicates that neither free ammonia nor the amide N from glutamine can serve as precursors of α-amino groups in HeLa cells grown in tissue culture.

On the other hand, the C:N ratio of the glutamine isolated from the cell proteins after the addition of doubly labeled glutamine to the tissue culture was the same as that of the added compound, even when unlabeled glutamic acid was also added. This is good evidence that the glutamine in the new protein arose directly from the glutamine in the medium. Also, when unlabeled glutamine was added to the medium with C^{14}-labeled glutamic acid and N^{15}-labeled ammonia, there was negligible labeling of the glutamine. There was, however, significant C^{14} in the glutamic acid isolated from the protein which suggests that the glutamic acid did not arise from glutamine which had been incorporated into the protein. When amide N^{15}-labeled glutamine was added to the medium the only component of the cell proteins, that was labeled, apart from glutamine itself, was the amide-N of asparagine.

These observations, together with the observation of Barry (*Biochem. J.* **63,** *669 (1956)*) that glutamine from blood is incorporated intact into the casein of milk, provide evidence that glutamine does not serve merely as an ammonia store, but is incorporated into proteins as an independent amino acid. The further observation that the ammonia released from glutamine was not incorporated into non-essential amino acids indicates that transamination is probably the major route of synthesis of non-essential amino acids in the HeLa cell line grown in tissue culture.

These two sets of observations may lead to a better understanding of the amino acid and nitrogen requirements of the intact animal. Although glutamine is not a dietary essential for higher species because of the ability of certain organs to synthesize it in adequate quantities (Meister, *loc. cit.*), it would appear that glutamine must be included among the non-essential amino acids that must be synthesized in the animal body from non-specific nitrogen sources.

NOTES

Letter to the Editor

Sir:

In your review entitled "Salt in Sheep Rations" (*Nutrition Reviews* **15,** *305 (1957)*) which mentioned our paper on the response of sheep on high grain rations to a salt supplement (*Aust. J. Agr. Res.* **8,** *83 (1957)*), the conclusion is drawn that the "results would tend to support the common belief that farm animals require salt to overcome the antagonistic effects of the high potassium content of animal feeds. I would not agree that the experiments reviewed support this as a general conclusion if grazing is included in "animal feeds."

All diets on which responses to salt have been obtained with livestock have contained a high proportion of grain, and grains, as is well known, are exceedingly poor sources of sodium. For example, wheat contains only about 20 parts per million. The available data indicate that grazing usually contains considerably more sodium than grain.

As indicated in the introduction in our paper, despite an ancient and continuing belief in the practice, there is as far as we are aware a complete lack of critical evidence that grazing sheep have *ever* responded to salt supplements; and many negative experiments have been recorded in this country. As regards cattle, to my knowledge the first and only unequivocal demonstration of a response by grazing animals to salt supplements has been recently provided by Rhodes (*Rhodesian Agricultural Journal* **53,** *969 (1956)*), although the response is apparently rather erratic (Rhodes, personal communication).

I would be most interested to learn of any other experiments indicating a response of grazing cattle to salt supplements, or any experiments indicating response by grazing sheep.

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Australia

Vitamin D and Atherosclerosis

The possibility that vitamin D in excess may be a causative factor in the development of arteriosclerosis has been discussed in some detail by C. O. de Langen and W. F. Donath (*Acta. Med. Scandinav.* **156,** *317 (1956)*). They have cited evidence from some of the early studies on vitamin D toxicity, indicating that degeneration and calcification occur in the aorta of rats and rabbits consuming diets high in vitamin D and that lesions have been found in both the media and the intima. They have also cited evidence that a high intake of vitamin D enhances the ability of dietary cholesterol to cause atherosclerotic lesions in rabbits.

As part of a long term reinvestigation of the relationships among vitamin D, vitamin A, cholesterol, mineral metabolism and atherosclerosis, they have studied the effect of large doses of vitamin D on the serum cholesterol levels of rabbits consuming a diet low in cholesterol. The rabbits were fed a diet containing oatmeal, wheat flakes, soybean meal, barleyhusk meal, yeast, cod liver oil and minerals. They maintained constant weight on this diet. One group received a weekly injection of 100,000 I.U. of vitamin D_2, another group received 100,000 I.U. of vitamin D_3, and a third received the same amount of vitamin D_2 with 200,000 I.U. of vitamin A.

Their results indicate that the serum cholesterol levels increased by 12 to 22 mg. per cent in each case in which an excess of vitamin D was administered. The amounts of vitamin D used were greatly in excess of those that would normally be consumed by man, but their speculation that a smaller dosage over a much longer time might produce similar effects must be considered. Their data are very limited and a number of the experimental details were not presented. Nevertheless, this indication that vitamin D may influence serum cholesterol level and the development of atheromatous lesions, should not be overlooked in attempts to

determine the significance of nutritional factors in the development of heart disease.

Nutrition and Pediatrics

The September–October number of the *American Journal of Clinical Nutrition* (**5,** *491,* (*1957*)) is devoted to a symposium entitled "The Interrelationship between Pediatric Disorders and Nutrition". The symposium, introduced by Dr. Paul György and Dr. L. E. Holt, Jr. who were guest editors for this number, consists of nine papers dealing with topics of current interest to both nutritionists and pediatricians.

The first two papers deal with general feeding problems in infants. One, "On the Evaluation of Artificial Feeding" by Mellander and Vahlquist of the University of Upsala, is a report of clinical observations on infants raised on breast milk or on formulas based on cows' milk, with particular emphasis on skeletal development. The other, "Failure to Gain Weight", by Holt, draws attention to the need for careful scrutiny of cases in which children who appear to consume adequate amounts of calories fail to grow at a normal rate.

Scrimshaw and his associates of INCAP in Guatemala discuss the problem of kwashiorkor (severe protein malnutrition) and its treatment. Lahey reviews in some detail observations relating to the importance of "Iron and Copper in Infant Nutrition." "The Pathogenesis of Idiopathic Hypercalcemia" is described by Stapleton and his associates of St. Mary's Hospital, London and the methods of treatment are appraised. The current status of water and electrolyte requirements is reviewed by Pratt.

Three papers deal with metabolic defects. Galactosemia is reviewed by Isselbacker, and phenylketonuria is reviewed by Armstrong and associates. Hunt discusses "Abnormally High Pyridoxine Requirement", pointing out that in a few cases this appears to be due to a metabolic abnormality.

Recent Nutrition Books

Protein Requirements. FAO Nutrition Studies, No. 16. Report of the FAO Committee. Food and Agriculture Organization of the United Nations, Rome, 1957. Pp. 52.

Technical Report of the Scientific Advisory Board for the Year 1956. Indian Council of Medical Research. The Job Press, Ltd., Kanpur Cambridge Printing Works, Delhi. Pp. 290.

Fluoridation of Public Water Supplies. By report of Commission of Inquiry, 1957. (The report is well documented and is clear in its endorsement of fluoridation). R. E. Owen, Government Printer, Wellington, New Zealand. Pp. 240.

Methods of Biochemical Analysis. Volume 5 (1957). Edited by David Glick. Interscience Publishers, New York City. Pp. 514. Price $9.50. Volume 4 (1957), pp. 372, price $8.50. Volume 3 (1956), pp. 447, price $9.50. Volume 2 (1955), pp. 476, price $9.50. Volume 1 (1954), pp. 531, price $9.50.

The Englishman's Food. A History of Five Centuries of English Diet. J. C. Drummond and A. Wilbraham. Revised and with a new chapter by Dorothy Hollingsworth. Johnathan Cape, London, January 1957. Pp. 482.

Calcium Metabolism. J. T. Irving, University of the Witwatersrand, Johannesburg, South Africa. John Wiley & Sons, Inc., New York. Pp. 177. Price $2.75.

Introduction to Protein Chemistry. Sidney W. Fox & J. F. Foster. John Wiley & Sons, Inc., New York. Pp. 459. Price $9.50.

Trace Analysis. Edited by John H. Yoe and Henry J. Koch, Jr. John Wiley & Sons, Inc., New York. Pp. 672. Price $12.00.

Proceedings of the Nutrition Education Conference. (April 1957). U. S. Dept. of Agriculture. Superintendent of Documents, Government Printing Office, Washington 25, D. C. Price $.45.

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